PATENT
1291-0186P

IN THE U.S. PATENT AND TRADEMARK OFFICE

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| Applicant: | Lars BRANDEN et al. | Conf.: | 8150 |
| Appl. No.: | 09/787,033 | Group: | 1632 |
| Filed: | May 14, 2001 | Examiner: | NGUYEN |

For: TRANSFER METHOD FOR SPECIFIC CELLULAR LOCALISATION OF NUCLEIC ACIDS

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Dr. S. I. Edvard Smith declare and say as follows:

1. I received my Ph.D. in Immunology in 1979 and my M.D. in 1980 from Karolinska Institutet in Stockholm, Sweden.

2. I am currently a Professor in Molecular Genetics at the Clinical Research Center, Karolinska Institutet, Huddinge University Hospital.

3. I have read the Office Action dated January 16, 2003 and the prior art cited therein. I now remark on the Examiner's rejection of the claims as being anticipated by, or obvious over the United States Patent of Felgner et al.(USP 6,165,720), hereinafter referred to as Felgner '720.

4. In the present claims, the simultaneous use of more than one single functional element (FE) is described. Felgner '720 does not deal with such simultaneous use of multiple ligands. Rather, Felgner '720 discloses a nuclear acid delivery

complex, which differs from the one claimed in the present application. In fact, Felgner does not deal with the simultaneous use of multiple ligands.

Felgner '720 uses PNA clamps, also referred to as bis-PNA. The DNA target sequence for such a clamp is made up of an AG-repeat as illustrated in Figure 3A of Felgner. Additional examples are found in Examples 1, 3 and 22 of Felgner '720. It is noted that in Example 22 of Felgner '720, the AG-opposite strand TC-repeat is presented.

To illustrate the advantageous results achieved by the present invention, the following experiments were carried out under my direct supervision and control. These experiments involve the use of the Human Immunodeficiency Virus 1 (HIV-1) TAT peptide and a novel branched peptide, each linked to a specific BE.

## Experiment
## Transfection efficiency using combinations of different FE-BE complexes

Figure 1 is a schematic representation of the experimental set-up. FE 1 is combined with different FE 2 (a, b, and c) to investigate the effect on the transfection efficacy.

***Brief Description of the Assay Conditions***

Hybridization

10 pmoles of 48 base pair oligonucleotide (TTT CAA TCG GAA CGG ATC GGA AGG GCC GAG CGC CAA TCG GAA CGG ATC) marked with a 6-

FAM fluorophore were hybridized to an FE-BE complex having either BE 1 (GAT CCG TTC CGA TTG) or BE 2 (GCG CTC GGC CCT TCC), which hybridized to the vector. FE-BE was added in two-fold excess over the number of vector BE target sequences. The hybridization was done either with BE 1 or BE 2 complexes separately, or in combination In the FE-BE complex, BE 1 was coupled to FE 1 (HIV-TAT peptide grkkrrqrrrppqc) whereas BE 2 was coupled to either FE 2a (which is an *acetylated* 8-armed lysine-branched peptide with the sequence kafrsaaq), FE 2b (which is an *non-acetylated* 8-armed lysine-branched peptide with the sequence kafrsaaq) or FE 2c (integrin-binding RGD peptide cyggrgdtp). The hybridization was carried out by heating the oligonucleotide and the FE-BE complex to 95° for 5 minutes followed by incubation at room temperature for two hours. Hybridization was verified with gel electrophoresis.

Transfection

Mononuclear cells were from purified human peripheral blood of a healthy donor and 1 million cells were used in each transfection The transfections were carried out in a 6-well plate and 10 pmoles of transfection complex (oligonuecleotide hybridized to FE-BE complex) were added to the cells. Cells not receiving any transfection complex, or receiving only oligonucleotide served as controls. After 90 minutes incubation at 37°, 5% $CO_2$, cells were washed one time with PBS (Phosphate Buffered Saline) and then treated with Trypsin for 5 minutes to

remove comp.exes bound to the cell surface in order to study only complexes t.ken up into the cells.

FACS Analys.s

After two washes with PBS, the cells were analyzed for transfectio. using a fluorescent activated cell sorter (FACS SCAN, Bectc.n Dickinson, Mountain View, CA). The fluorescent signal fron cells not receiving any transfection complex or oligonucleo.ide was subtracted from the measured values.

Results

The re.ults are shown in Figure 2. The use of combinations of two diffe.rent FEs clearly increases the transfection efficacy.

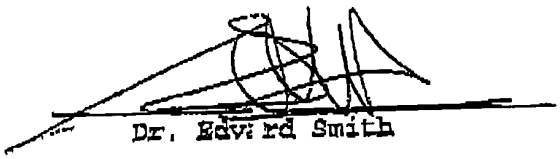
Discussion

A revie.w of the results as displayed in Figure 2 reveals that when :wo different FEs are utilized, the transfection efficiency i.s greatly increased compared to the use of the FEs individually In fact, a close inspection of Figure 2 reveals that the use of FE1, FE2a, FE2b, and FE2c individually resulted in values ra.ging from 0.5 to approximately 1.1 percent of cells transfected. In contrast, when two different FEs are used in combination, for instance, FE1+2a, FE1+2b and FE1+2c, the percentage o: cells transfected rises to between 4 and 7 percent. It is thus e.ident that the use of both FE-BE complexes gives an uptake of th: vector that is greater than the sum of the uptake of vector w.en each FE-BE is used separately. This greatly

increased transfection efficiency is totally unexpected in view of the disclosure of Felgner '720.

Based on the lack of disclosure in Felgner '720 of more than one functional element, no anticipation nor obviousness exists. Moreover, as shown in the experimental data, when more than one functional element-binding element complex is utilized, there exists a level of uptake of the vector which is greater than the sum of the uptake of the vector when these complexes are used separately. Such results are unexpectedly superior to the disclosure of Felgner '720, thus any case of obviousness is rebutted.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under 18 U.S. Code 1001 and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.



Dr. Edward Smith

05/06/03

Date